A Model of the Development of the Brain as a Construct of the Thyroid System

Kembra L. Howdeshell

Wildlife and Contaminants Program, World Wildlife Fund, Washington, DC, USA

Thyroid hormone is essential for normal brain development. However, little is known about the molecular and cellular mechanisms that mediate thyroid hormone action on the developing brain or the developmental events selectively affected. Consequently, although a large number of environmental chemicals interfere with the thyroid system, there are few neurodevelopmental end points to recruit for toxicological studies. Therefore, my goal here is to review what is known about the relative timing of normal brain construction and thyroid system development, with special focus on the period of *in utero* development in humans and the comparable developmental period in laboratory rats. These data are presented as a timeline to aid in the identification of thyroid-sensitive end points in brain development and to highlight important data gaps. I discuss the known influence of certain synthetic chemicals on the thyroid system and include a brief review of the effects of developmental exposure to chemicals on thyroid system function. The relationship between the thyroid hormone and retinoic acid systems, as well as the thyroid hormone sensitivity of the developing cochlea, is also discussed. *Key words:* brain, development, endocrine disruptors, hearing loss, heart, retinoic acid, thyroid, xenobiotic. *Environ Health Perspect* 110(suppl 3):337–348 (2002).

http://ehpnet1.niehs.nih.gov/docs/2002/suppl-3/337-348howdeshell/abstract.html

In response to the mandate of the U.S. Environmental Protection Agency (U.S. EPA) to develop a set of screens and assays to test chemicals for their possible estrogenic or other endocrine effects, a workshop was convened in June 1997 to assist the U.S. EPA in developing tests available to detect chemicals that might interfere with the thyroid system. The "Workshop on Screening Methods for Chemicals that Interfere with Thyroid Hormone Action, Function and Homeostasis" was convened at the Nicholas School of the Environment, Duke University, Durham, North Carolina by the Chemical Manufacturers Association, the U.S. EPA, and the World Wildlife Fund (*1*). The workshop panelists agreed that there were several assays currently in use in the medical and toxicological communities that could detect various mechanisms of thyroid system disruption and suggested using a combination of such tests to develop an assay. They also pointed out that no environmental contaminant had been identified as a thyroid hormone receptor (TR) agonist or antagonist. They recommended the use of thyroid histopathology and/or quantification of serum thyroid hormone to reduce false positives and negatives, and suggested that serum thyroid hormone concentrations could provide an initial screen in mammals. Yet, despite the counsel of the workshop participants, little progress has been made toward the development of assays to test chemicals for their impact on the thyroid system.

My purpose here is to expand upon the results of the 1997 Durham workshop with two main goals. The first goal is to produce an inventory of data on the development of the thyroid system and concurrent development of the brain to provide a visual aid for the identification of thyroid-sensitive periods of brain maturation. The two visual aids are designed in the form of timelines based upon a literature review of scientific journals and medical reference materials: one timeline for human development from conception to birth and a second timeline for the laboratory rat from conception to postnatal day (PND) 20, the comparable developmental time period relative to humans. The second goal is to discuss what is known about how man-made chemicals can interfere with the normal production, transport, metabolism, and excretion of thyroid hormone during development, and their known impact on the thyroid system during development. Perturbation of the interaction of the thyroid and retinoic acid (RA) systems is also briefly reviewed. Finally, thyroid hormone sensitivity of the nervous system derivative of the inner ear, the cochlea, is discussed as a potential end point of thyroid hormone disruption.

The literature review presented below indicates that thyroid hormone is potentially important from the very earliest stages of development—from the mature oocyte and throughout gestation. Thyroid receptors, the proteins necessary for thyroid hormone signal transduction, have also been detected during these periods, and there is evidence of structural and functional defects in brain development when thyroid hormone is absent or in short supply. The timing of the onset of fetal thyroid function and the acquisition of regulatory mechanisms controlling thyroid hormone action in the fetal brain indicate that thyroid hormone of maternal origin plays an important role in early developmental events in the brain. Thus, by superimposing the timing of brain development onto development of the thyroid system, it becomes clear that any environmental chemical that affects maternal thyroid status or that directly affects thyroid hormone action may exert very specific effects on the complex mechanisms underlying normal brain development.

Developing a Timeline Model of Thyroid System and Brain Development

Human Thyroid System Development

The timeline begins with a review of the basic development of the thyroid system from conception until birth in humans (Figure 1).

Thyroid gland development. Development of the human thyroid gland begins in the third week of gestation (*2*) (Figure 1, grid 1). On gestation day (GD) 16–17, the embryonic thyroid is visible as an outgrowth in the floor of the primitive buccal cavity (the site of the future pharynx). Additional lateral contributions to the embryonic thyroid originate from a portion of the fourth pharyngeal pouches; these lateral contributions are the origin of the C-cells, which produce calcitonin. The thyroid gland tissues merge and migrate to their final location by GD45–50. By GD70, the thyroid gland is well developed; it begins concentrating

This article is part of the monograph *Impact of Endocrine Disruptors on Brain Development and Behavior.*

Address correspondence to K.L. Howdeshell, Wildlife and Contaminants Program, World Wildlife Fund, 1250 24th Street NW, Washington, DC 20037-1193 USA. Telephone: (202) 778-9736. Fax: (202) 530-0743. E-mail: kembra.howdeshell@wwfus.org

I thank T. Colborn, T. Zoeller, and K. Thayer for their suggestions and constructive criticism of this review. I thank L. Carson and R. Bauer for their editorial assistance in the preparation of this manuscript for publication. Funding for this project was provided by the New York Community Trust and The Windslow Foundation.

Received 8 January 2002; accepted 17 April 2002.

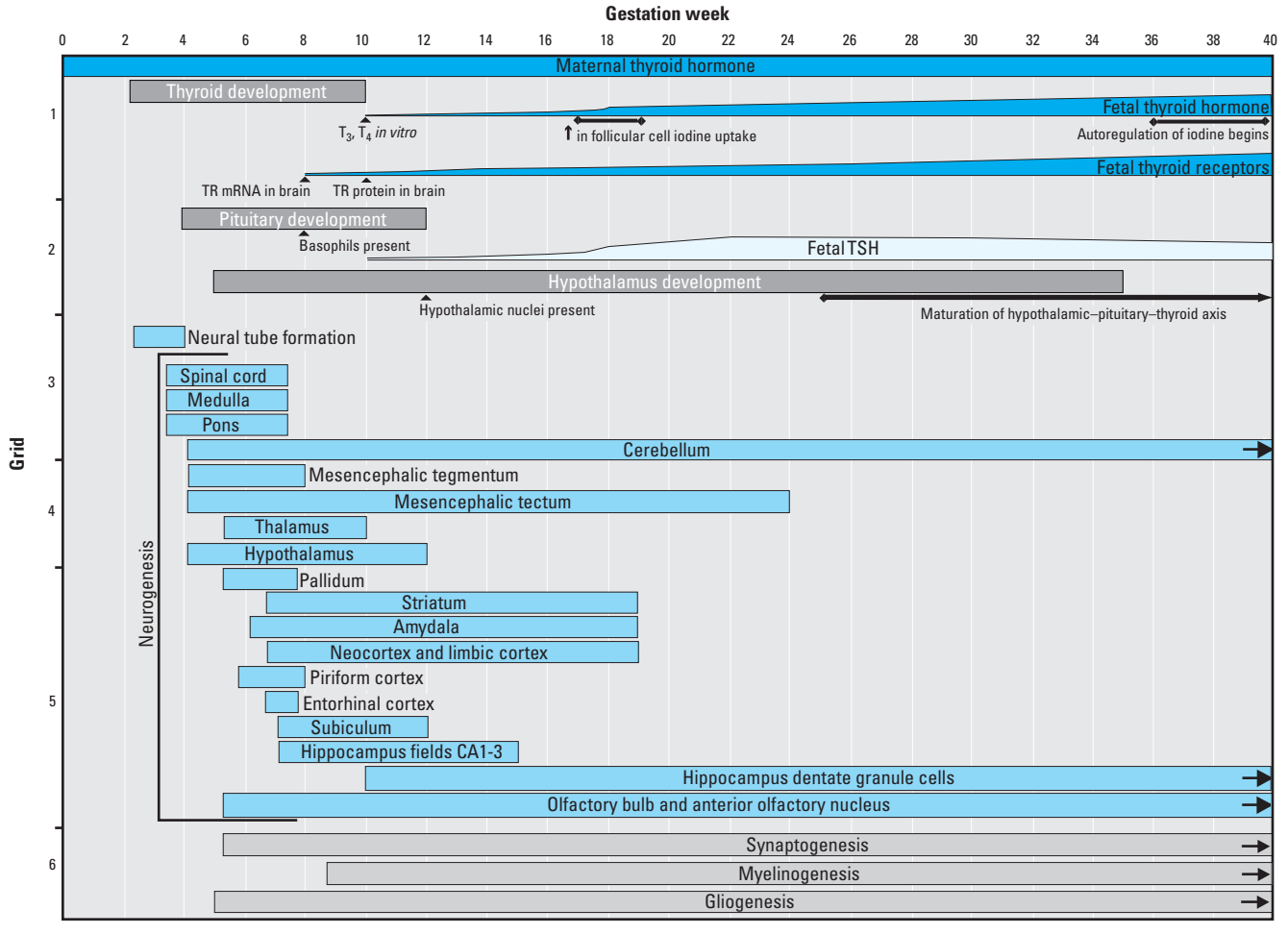

Figure 1. Timeline of human thyroid system and brain development from conception to birth. Estimation of neurogenesis adapted from Bayer et al. (*49*).

iodide and producing thyroid hormone at this time (*3*). Thyroglobulin (TBG), the precursor protein upon which thyroid hormone is produced and stored, is present as early as GD29 in thyroid follicle cells (*2*). As the thyroid follicle cells mature, TBG levels are detected in fetal serum by gestation week 11 and increase through gestation (*4*).

Maternal/fetal thyroid hormone. Thyroid hormone is produced from iodinated tyrosine residues to form 3,5,3′,5′-tetraiodothyronine (thyroxine [T_4]) and 3,5,3′-triiodo-L-thyronine (T_3). T_4 is the most abundant product of the thyroid gland in circulation and is peripherally deiodinated to T_3, the bioactive form of thyroid hormone. The bioavailability of thyroid hormone is tightly controlled by the binding of the hormone to serum-binding proteins (i.e., TBG, transthyretin, and albumin), which maintains a fixed percentage of free (not bound to serum-binding protein) thyroid hormone available to elicit thyroid action (*5*). During pregnancy, there is increased peripheral metabolism of free thyroid hormone; the pool of serum protein-bound thyroid hormone also increases during this time to maintain the proper level of free hormone (*6*). Thus, the total (free and bound-to-serum protein) levels of maternal T_4 and T_3 increase during pregnancy to meet increased demand, while the maternal level of free T_4 remains constant (*7,8*) (Figure 1, grid 1).

It had long been thought that fetal exposure to maternal thyroid hormone was nonexistent or limited, because maternal T_4 is largely converted to reverse T_3 (an inactive form) in the placenta (*8*). However, in 1989, Vulsma et al. (*9*), demonstrated that maternal T_4 did reach the fetus by observing infants with either thyroid agenesis or a total organification defect, a mutation abolishing the ability to iodinate thyroid protein. In infants with these conditions, the maternal circulation provides 35–70 nmol/L of serum T_4 (20–44% of the normal levels) as measured within the first 4 weeks of postnatal life; normal serum T_4 levels during this time period are 80–170 nmol/L serum T_4. Maternal–embryo transfer of thyroid hormones has been detected in embryonic coelomic fluid (total T_4 = 961 ± 193 pmol/L or 747 ± 150 pg/mL; total T_3 = 33 ± 13 pmol/L or 18.5 ± 7.3 pg/mL) and amniotic fluid (20 ± 5 pmol/L or 16.0 ± 3.8 pg/mL; total T_3 was below the detection limit of the assay) at 5 to 11 weeks of gestation (*7*). At term, total and free serum levels of fetal T_4 are similar to maternal values, whereas maternal serum T_3 levels (total and free) continue to be two to three times greater than fetal free T_3 levels (*4*). Recent studies indicate that the maternal contribution of thyroid hormone is critical for proper *in utero* brain development, as evidenced by impaired psychomotor development and visuospatial processing in offspring born to mothers with low serum levels of free T_4 (*10–13*).

As mentioned above, fetal thyroid hormone production begins at approximately 10 weeks of gestation in *in vitro* organ culture of human thyroid tissue (*3*) (Figure 1, grid 1). In an *in vivo* study, Bernal and Pekonen (*14*) detected T_3, but not T_4, in homogenized human brain tissue at 10 and 12 weeks of gestation. The tissue concentration of free T_3 is consistently higher in the

nuclear cell fraction than in the cytoplasmic fraction of 10–13 gestational week human brains, thus providing evidence for a gradient of increased free T_3 concentration in the cell nuclei versus the cytosol (*15*); a similar free T_3 concentration gradient from plasma to cytosol to nuclei has been reported in the adult rat brain (*16*).

Brain tissue levels of T_4 exceed T_3 levels at 16 weeks of gestation and beyond (*14*). Thorpe-Beeston et al. (*4*) reported increasing levels of free T_4 and T_3 in cord blood from gestation weeks 12–40. Increased thyroid hormone production is due, in part, to an increase in thyroid gland follicular cell uptake of iodide from gestation weeks 18–20 and subsequent maturation of the hypothalamic-pituitary–thyroid axis (see below). The autoregulation of iodide by the fetal thyroid gland develops during 36–40 weeks of gestation (*17*).

Thyroid hormone receptors. Thyroid hormone elicits its effect via binding to the TR, a nuclear transcription factor (*18*). The ligand-bound thyroid receptor dimerizes with a second TR and binds to a thyroid response element on the DNA to elicit gene expression. Thyroid hormone receptors are products of two genes: c-*erb*Aα encoding the isoforms TRα1 and TRα2, and c-*erb*Aβ encoding the isoforms TRβ1 and TRβ2. All TR isoforms bind both T_4 and T_3, with the exception of TRα2; TRα2 does not bind hormone due to a mutation in its ligand-binding domain. The abundance of TR isoforms is thought to allow for the tissue specificity of thyroid hormone action. Additionally, the TR can bind with the retinoic X receptor (RXR) of the RA system, which will be described later in this article.

Thyroid hormone receptors appear before the initiation of fetal thyroid function, and they increase in association with the increase in fetal thyroid hormone production (Figure 1, grid 1). Bernal and Pekonen (*14*) identified TR protein in human fetal brain tissue at 10 weeks of gestation. More recently, the expression of TRα1 and TRβ1 mRNA was detected in human whole-brain samples as early as 8 weeks of gestation (*19*). TRα1 mRNA increased 8-fold from 8 through 13.0 weeks of gestation, whereas expression of TRβ1 mRNA increased at 13.9 weeks of gestation after low-level expression from 8 to 10 weeks of gestation. Similarly, Kilby et al. (*20*) detected the expression of all TR isoforms (TRα1, TRα2, TRβ1, TRβ2) in cerebral cortex samples from 10 to 16 weeks of gestation. In fetal cerebral cortex and cerebellum, only TRα1 and TRα2 protein was detected in the first trimester samples (11–13 weeks of gestation), while receptor protein of all four TR isoforms was detected in the second (15–25 weeks of gestation) and third trimester (26–40 weeks of gestation) samples (*20*). TR mRNA and protein have been detected in the human brain at the earliest time points studied, which suggests that TR may be present even earlier in brain development. Interestingly, the mRNA of all four TR isoforms are expressed in mature human oocytes and granulosa and cumulus cells (*21*), thus indicating that thyroid hormone may influence development from the time of conception and early embryogenesis.

Human Hypothalamic–Pituitary–Thyroid Axis Development

Thyroid hormone production is regulated by a sensitive feedback system between the hypothalamus, pituitary, and thyroid gland. Thyrotropin-releasing hormone (TRH) is secreted from the hypothalamus, which acts upon the pituitary to stimulate the release of thyrotropin (also called thyroid-stimulating hormone [TSH]). TSH then acts upon the thyroid gland to initiate thyroid hormone production. Increased circulatory levels of thyroid hormone then provide negative feedback on the hypothalamus and pituitary to inhibit T_4 and T_3 production, thus maintaining proper levels of hormone availability.

Pituitary gland development. Although the neuroendocrine control of thyroid hormone function develops much later in fetal development, the pituitary and hypothalamic structures begin to develop within weeks of thyroid gland emergence (*22,23*). The anterior pituitary gland is derived from Rathke's pouch, which first appears on gestation week 4 as an outgrowth of the primitive buccal cavity (Figure 1, grid 2). By gestation week 5, the anterior pituitary is connected to a derivative of the cerebrum that will become the posterior pituitary. Cell differentiation in the pituitary is initiated by 7–8 weeks of gestation. Basophils, which include thyrotropes (thyrotropin-secreting cells) and gonadotropes (gonadotropin-secreting cells), are detected in the pituitary by 8 weeks of gestation (*22*), and large numbers of thyrotropes are present by 12–13 weeks of gestation (*23*). The development of the hypophyseal portal system, the vasculature connecting the pituitary to the median eminence, is concurrent with pituitary gland development; capillaries of the hypophyseal portal system have been detected as early as 7–8 weeks of gestation.

Thyroid-stimulating hormone. TSH is first detected in the fetal human pituitary by 10–12 weeks of gestation (*22*) (Figure 1, grid 2). Fetal pituitary TSH levels are relatively low until gestation weeks 16–20, after which they increase to parturition. Fetal serum levels of TSH are first observed at 10–12 weeks of gestation. Fetal serum levels reach peak concentrations around gestation weeks 22–30, then decrease slightly until birth (*22,23*). The decrease in TSH before birth is thought to be, in part, in response to the onset of the negative feedback system of the maturing hypothalamic–pituitary–thyroid system.

Hypothalamus development. The hypothalamus develops from the ventral portion of the diencephalon and is visible by 5 weeks of gestation (*22,23*) (Figure 1, grid 2). Hypothalamic nuclei and the fibers of the supraoptic tract are detectable at 12–14 weeks of gestation. The hypothalamic nuclei and their processes continue to mature through 30–35 weeks of gestation. The hypothalamo–hypophyseal portal system, the vasculature connecting the hypothalamus with the pituitary via the median eminence, is observed in human fetuses as early as 11.5 weeks of gestation (*24*). This circulatory system, also referred to as the primary portal system, continues to mature through late gestation.

Thyrotropin-releasing hormone. TRH is detected in fetal whole-brain samples by 4.5 weeks of gestation (*25*). The levels of TRH detected this early are likely of maternal origin, as the fetal hypothalamus is just beginning to develop and maternal TRH is able to cross the placental barrier (*2*). TRH is detected in fetal hypothalamus extracts as early as 8–11 weeks of gestation (*25,26*). The hypothalamic–pituitary–thyroid axis begins to mature during the second half of gestation (*22*). The fetal pituitary can respond to TRH as early as 25 weeks of gestation; this is evidenced by an increase in fetal TSH secretion upon maternal administration of TRH, as measured by a cordocentesis procedure on normal pregnancies during 25–37 weeks of gestation (*4*).

Rat Thyroid System Development

Thyroid gland. The developing rat thyroid gland is first visible on GD9 as an endodermal thickening in the primitive buccal cavity (*27*) (Figure 2, grid 1). The thyroid gland is well developed and positioned in the fetal thyroid gland contains TBG and is capable of concentrating iodide. *In vitro* cultures of rat thyroid indicate its ability to synthesize thyroid hormone at least as early as GD20 (*3*).

Maternal and fetal/neonatal thyroid hormone. Maternal transfer of thyroid hormone to the embryo/fetus has been verified in the laboratory rat (Figure 2, grid 1). Thyroid hormone is detected in rat embryotrophoblasts as early as GD9 (total T_4 = 4.46 ± 1.04 ng/100 mg and

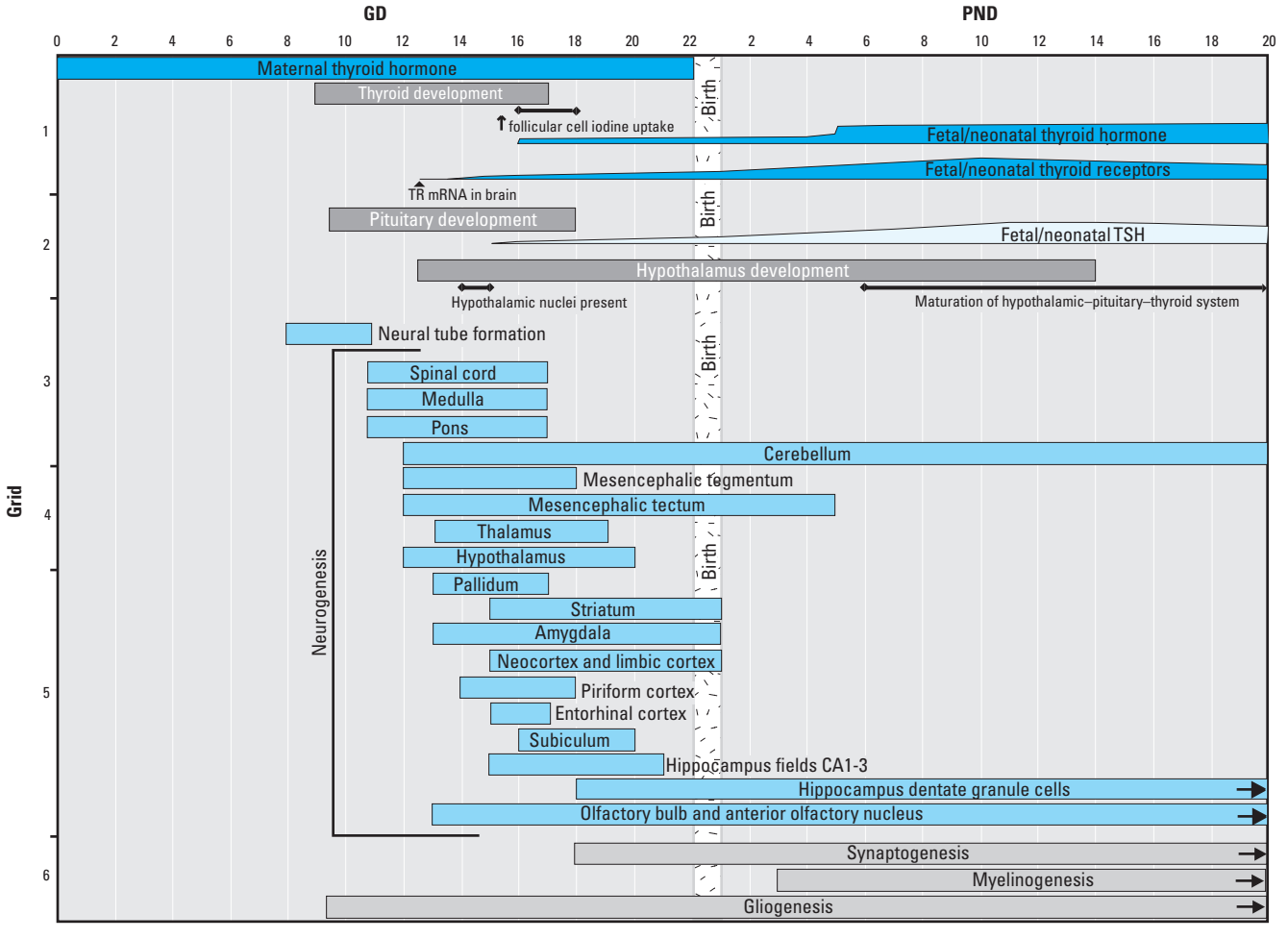

Figure 2. Timeline of rat thyroid system and brain development from conception to PND20. Conception = GD0 and birth = PND1. Timing of neurogenesis adapted from Bayer et al. (*49*).

total T_3 = 0.18 ± 0.02 ng/100 mg (*28*). Woods et al. (*29*) reported that rat embryotrophoblasts (GD9–10) contained 21% of the T_4 and 54% of the T_3 of the maternal dose of radiolabeled T_4 or T_3 1 hr after administration. Versloot et al. (*30*) demonstrated that even marginal maternal iodide deficiency decreases the availability of T_4 for the fetus in the rat. Normal maternal serum T_4 levels are essential to the maintenance of fetal serum T_4 available for local conversion to T_3 (*31*).

The total T_4 and T_3 concentrations in rat fetuses increase dramatically from GD18 until birth because of maturation of hormone synthesis of the fetal thyroid gland (*22,32*) (Figure 2, grid 1). Total T_4 and T_3 tissue levels of GD18 rat fetuses measured 190 ± 38 pg/g and 33 ± 4 pg/g, respectively (*28*). By GD21, fetal plasma levels had reached 0.4 ± 0.03 µg/dL total T_4 and 17 ± 4 ng/dL total T_3. After birth, neonatal serum levels of total T_4 rise dramatically from 1.0 µg/dL at PND5 to a peak level of 6 µg/dL at PND17 (*33*). Neonatal serum levels of total T_3 rise in parallel to total T_4 with a peak serum value of 108 ng/dL reached on PND28. Free serum T_4 and T_3 levels follow a pattern similar to the total serum values (*22*). The serum levels of T_4 and T_3 reach adult values at approximately PND40.

Fetal/neonatal thyroid hormone receptors. Fetal TRs are present early in fetal rat brain development (*34*). TRβ1 and TRβ2 isoforms were detected as early as GD12.5 in the rat otic vesicle in the fetal brain, the portion of the embryonic inner ear that develops into the cochlea (*35*) (Figure 2, grid 1). Falcone et al. (*36*) detected TRα1, TRα2, and TRβ1 protein at GD14 in rat brains, particularly in regions of neuronal and glial cell nuclei. Levels of TR in the fetal brain increase 3-fold from GD14 to GD16–17, then remain steady until birth (*34*). Following birth, TR mRNA and protein levels increase through the second week of postnatal life, reaching peak mRNA concentrations on PND10 and protein concentrations on PND15 (*37,38*).

There is evidence that the TRα and TRβ isoforms are selectively expressed in different brain regions during development and may therefore mediate different functions. For example, Bradley et al. (*39*) found that the TRβ1 mRNA is selectively expressed in the ventricular zone of the early cortex where cells undergo proliferation and early fate specification. However, as these cells leave the cell cycle and begin to differentiate, they migrate away from the ventricular zone, cease expressing TRβ1, and begin expressing TRα1. Likewise, Strait et al. (*40*) observed a selective pattern of TRβ1 and TRα2 expression in cerebellar sections of neonatal (PND4) and adult (PND60) rats. TRβ1 protein was strongly localized to the nuclei of Purkinje cells and weakly present in the nuclei of granule cells, whereas TRα2 was detected only in the nuclei of granule cells. Neither TR isoform was detected in the neuronal axons or glial cells. In contrast, Leonard et al. (*41*) concluded that TRα2 is selectively expressed in astrocytes, but not neuronal cells. Although studies using transgenic mice and targeted deletion/mutations have helped elucidate some of the physiological functions of specific TR isoforms (*42*),

distinct roles for the TR isoforms in specific neurodevelopmental events have yet to be identified.

Rat Hypothalamus–Pituitary–Thyroid Axis Development

Pituitary development. The first sign of rat anterior pituitary development is the formation of Rathke's pouch, which appears on GD9.5 (*27*) (Figure 2, grid 2). By GD16, the pituitary is well developed and secretory granules are present (*22*). The number of granule cells increases from GD18 through the end of the first postnatal week. The posterior pituitary is well developed by GD18. The pituitary portal system develops in concert with the pituitary gland and is well developed by GD17.

Thyroid-stimulating hormone. Pituitary TSH mRNA expression begins on GD15 (*43*), while TSH protein levels are first reported at GD17 measuring 24.8 ± 2 ng/pituitary (*44*) (Figure 2, grid 2). Fetal pituitary levels climb to 162.0 ± 9.64 µg/pituitary for males and 194.5 ± 9.14 µg/pituitary for females. Serum levels of TSH are 20 ng/100 µL at birth and increase to maximum postnatal serum levels by PND7–8 (40 ng/100 µL TSH) (*22*). Pituitary levels of TSH increase from 170 ng/µg protein at birth to approximately 1 µg/µg protein by PND10–12. The levels of pituitary and serum TSH slowly decrease from PND14–16 until reaching adult levels at PND40 (*33*).

Hypothalamus development. The developing hypothalamus is first visible on GD12.5 as the diencephalon differentiates into the hypothalamus, epithalamus (which comprises the pineal gland), and the thalamus (*27*) (Figure 2, grid 2). By GD14–15, hypothalamic nuclei are present and there is continued ingrowth of axons into the median eminence (*22*). The hypothalamus and median eminence are nearly morphologically complete by the third postnatal week. The vasculature of the hypothalamo–hypophyseal portal system is known to reach the median eminence late in the first postnatal week, with continued development of the portal system occurring through postnatal weeks 5–6.

Thyrotropin-releasing hormone. TRH mRNA can be detected as early as GD14 in neurons of the rat fetal hypothalamus (*45*). By GD15, TRH mRNA is detected in the developing paraventricular nuclei of the hypothalamus. At birth, TRH mRNA was detected in all areas of the brain known to express it in adulthood. Adult TRH mRNA expression patterns are present by PND22. TRH is produced in low levels (6.0 ± 0.5 pg/mg) in the rat hypothalamus as early as GD16 and increases approximately 3-fold by GD20 (*46*). TRH levels increase to adult levels by PND17–29, then decrease transiently between PND31–41; adult levels are once again reached at PND50 (*33,44*). Although TRH is present in the hypothalamus in late gestation, it does not appear to influence the hypothalamic–pituitary–thyroid axis until the second week of postnatal life (*22,47*).

Mammalian Brain Development (Human and Rat)

Central nervous system embryogenesis begins with formation of the neural tube. This process begins around 2 weeks of gestation in humans (GD8–9 in rats), with the neural plate developing from ectoderm as directed by the notochord (*27,48*) (Figure 1, grid 3 human; Figure 2, grid 3 rat). As the neural plate invaginates and begins to fuse, neural crest cells are derived from the ectoderm at the interface closing neural tube and skin ectoderm. The neural crest cells are progenitors of several components of the nervous system, including the sensory ganglia of the spinal and cranial nerves, the Schwann cells (neuroglial cells covering the peripheral nerves), the meninges (connective tissue covering of the brain), and other derivatives. The neural tube closes in a caudal to rostral pattern with hindbrain (rhomencephalon) regions forming before forebrain (telencephalon) regions. In humans, the neural tube formation begins at approximately GD18 (rats ~GD9–9.5) and is complete by GD26–28 (rats GD10.5–11) (*48*).

Bayer et al. (*49*) estimated the timing of human fetal brain neurogenesis based upon extensive histological observation of rat fetal and neonatal brain development compared with anatomical drawings and photographs of human fetal brain development. The initiation of neurogenesis in the developing brain proceeds in a caudal to rostral direction beginning with the spinal cord and the hindbrain derivatives, including the myelencephalon (medulla) and metencephalon (pons and cerebellum) (Figure 1, grid 3 human; Figure 2, grid 3 rat). Neurogenesis subsequently begins in the mesencephalon (tectum and tegmentum) and diencephalon (thalamus and hypothalamus) (Figure 1, grid 4 human; Figure 2, grid 4 rat). The telencephalon is the last of the brain regions to begin to develop; it encompasses the cerebral structures, including the basal ganglia (striatum and pallidum), amygdala, and cerebral cortex (neocortex, limbic and piriform cortices, hippocampal regions, and the olfactory bulb) (Figure 1, grid 5 human; Figure 2, grid 5 rat). Neurogenesis in the cerebellum, hippocampus dentate granule cells, and the olfactory bulb continue into postnatal life in humans and rats (*49*). Recent studies in rodents and primates suggest that regions of the hippocampus and olfactory bulb continue to undergo neurogenesis well into adulthood (*50,51*).

The initiation of migration of neurons is concurrent with the period of neurogenesis and is usually completed within days in the rat and within weeks in humans (*49*). Differentiation is a process that generally begins after a brief pause following migration (*49*); however, cell fate determination may begin as early as migration (*48*). In humans, synaptogenesis is reported to begin as early as gestation month 2 and continues into postnatal life, with a peak in the first year of life (*52*) (Figure 1, grid 6). In rats, synapse formation is detected as early as GD18 in the substantia nigra (*53*), with the majority of synapse formation occurring after birth (*54,55*) (Figure 2, grid 6). Myelin production first appears around gestation month 5 in humans and continues well into postnatal life (*52*) (Figure 1, grid 6). Myelin protein mRNA is clearly expressed in cranial nerve VIII (*56*) by PND2, and in the cerebellum and mesencephalon by PND10, followed by expression in more rostral brain regions later in rat development (*57*) (Figure 2, grid 6).

Thyroid Hormone Influence on Brain Development

Thyroid hormone is essential for brain development (*37*, *58–62*); the following section is a brief summary of some of thyroid hormones' effects on neuronal proliferation, migration, synaptogenesis and myelination. For example, in the cerebellum, thyroid hormone plays an important role in granule cell proliferation and survival. Granule cell proliferation, as measured by ^{3}H-thymidine, is enhanced by thyroid hormone and suppressed by hypothyroidism (*63*). In addition, granule cell apoptosis, as measured by terminal deoxynucleotydl transferase-mediated dUTP nick end-labeling, was increased 8-fold in hypothyroid animals (*64*). Direct effects of thyroid hormone on the expression of genes encoding growth factors and antiapoptotic genes may mediate these effects. Neveu and Arenas (*65*) found that hypothyroidism increased the abundance of mRNAs encoding nerve growth factor and neurotrophin 4/5 and severely reduced the expression of the mRNAs encoding neurotrophin 3 and brain-derived neurotrophic factor in the cerebellum. However, there were no effects of thyroid hormone on levels of the full-length tyrosine kinase B and C receptor mRNAs. In addition, Muller et al. (*66*) found that

Bcl-2 (B-cell lymphoma 2 protooncogene) administration prevented the increased apoptosis in the cerebellum induced by hypothyroidism.

Thyroid hormone availability influences neuronal migration, as evidenced by decreased whole-brain weight and densely packed neurons in the hypothyroid rat cerebellum (*63,67*). Many genes involved in the process of neuronal migration are sensitive to thyroid hormone (*62*). For example, Alvarez-Dolado et al. (*68*) reported that thyroid hormone increases mRNA expression and translation of *reelin*, which encodes a large extracellular protein. Conversely, hypothyroid conditions significantly reduce the abundance of *reelin*-containing cells and the intensity of signal from each cell. Neural migration is also influenced by thyroid hormone effects on astrocytes, a glial cell type. Astrocytes secrete laminin, a key guidance signal for the migration of neurons (*69*). In the developing rat cerebellum, thyroid hormone deficiency delays the expression of laminin, and total laminin concentration is 35% less intense than in controls (*70*).

Synaptogenesis is negatively affected in the hypothyroid brain due to the decrease in axonal length and branching of neurons, thus reducing neuronal connections. Hypothyroidism decreases neural axonal density and growth in the cerebral cortex (*71*) and reduces the dendrite branching of Purkinje cells in the cerebellum (*72*), pyramidal cells in the cerebral cortex (*73*), and cortical neurons of the corpus callosum (*74*). Rami and Rabie (*75*) report a delay in hippocampal synaptogenesis under hypothyroid conditions, based on measurements of synaptophysin, a marker protein of mature synaptic vesicles. In the cerebellum, mature granule cells in the internal granular layer express a synaptotagmin-related gene 1 (*Srg1*) (*76*). Thyroid hormone upregulates *Srg1* mRNA, whereas hypothyroidism decreases its expression. Finally, a reduction in myelination is also a common phenotype of the hypothyroid neonatal brain. The decrease in myelination is due, in part, to the decrease in length and branching of neural outgrowths as previously described. Thyroid hormone also influences myelination by stimulating the differentiation of oligodendrocytes, which are the glial cells responsible for the production of the myelin proteins (*77*). Ibarrola and Rodríguez-Peña (*57*) report that hypothyroidism causes delayed expression of four major myelin protein genes: myelin basic protein (MBP), proteolipid protein (PLP), 2′:3′-cyclic nucleotide 3′-phosphodiesterase, and myelin-associated glycoprotein; the delayed expression of such genes leads to an overall decrease in myelin protein production. In contrast, hyperthyroidism increases the MBP and PLP mRNA, and this effect is likely due to an increased abundance of mature oligodendrocytes (*77*).

Gliogenesis and its responsiveness to thyroid hormone has most often focused on the development and maturation of the astrocytes and oligodendrocytes, which develop well after the bulk of neurogenesis is complete (*48,77*). However, radial glial and microglial cells are present much earlier in brain development and are also influenced by thyroid hormone. Radial cells provide a framework for neuronal migration (*78,79*) and are the precursor cell line to astroglial cells (*80*). In mice, radial glial cells are present in the developing neural tube as early as GD9.5 (*81*) and by estimated GD11 in rats (*27*). Microglial cells are macrophage cells, which phagocytize dead cells and cellular debris following apoptosis during brain development. In mice, microglia progenitor cells are derived from the yolk sac and are present in future brain tissue by GD8 and mature microglial are detected by GD13 (*82*); in rat the estimated time points are GD9.5 and GD14, respectively (*27*) (Figure 2, grid 6). Radial and microglia are also present very early in human gestation (*52,83*) (Figure 1, grid 6). Prenatal hypothyroidism inhibits the maturation of radial glial cells in the CA1 region of GD21 rat hippocampus, as measured by the abundance of glial fibrillary acidic protein (GFAP) (*84*). A deficiency of GFAP during development inhibits the branching necessary for astrocyte–neuronal connections and decreases the long-term potentiation capacity, a factor essential to learning and memory (*85*). Microglial cells express the TRα1 and TRβ1 isoforms and are influenced by thyroid hormone (*86*). Microglial cell density and process formation are depressed in the parietal cortex of neonatal rats exposed to hypothyroid conditions from GD16 through lactation (*86*). In contrast, hyperthyroidism induced by T_3 injections to euthyroid and prenatally hypothyroid rat pups increased microglial cell survival and branching of the developing axonal fiber tracts of the corpus callosum. Although Lima et al. (*86*) identify the first postnatal week in rats as a critical window for thyroid hormone influence, they suggest that maternal thyroid hormone could be influencing the early development of fetal microglial cells. The fact that both radial glial and microglial cells are negatively affected by hypothyroid conditions indicates that thyroid hormone may influence very early stages of brain development.

The complexity of development within each brain region suggests that investigations of thyroid hormone influence on the brain should be conducted with as much specificity to region as possible. For example, different cell populations within each area of the brain often have their own unique timeline for birth and maturation (*49,52*). Neurogenesis of the Purkinje and granule cells populations occurs at different times in the cerebellum; Purkinje cells emerge between GD13–15 in the rat (human gestation weeks 5.3–7.0), whereas the granule cells do not begin to appear until PND4 in the rat (human gestation week 24.0) (*49*). Similarly, the peak proliferation of granule cells in the hippocampus occurs after birth, whereas maximal neurogenesis of the hippocampal CA3 neurons occurs between GD17 and GD20 (*49*). Different regions of the brain also have particular developmental schedules. As demonstrated in Figures 1 and 2, neurogenesis is initiated in hindbrain derivatives (i.e., medulla and pons) well before it begins in forebrain derivatives (i.e., neocortex and hippocampus) in both humans and rats (*49*). Similarly, Herschkowitz et al. (*52*) reviewed the timing of brain development in humans and found the onset of myelination occurred at specific times for the various brain areas. For example, myelination of the spinal nerve roots begins around gestation month 5, whereas myelination of the auditory cortex does not begin until gestation week 9. Likewise, myelinogenesis in the rat brain occurs in a regional pattern (*87*). Myelination of the vestibulo–cochlear nerve (cranial nerve VIII) is evident by PND2 (*56*), whereas onset of myelin protein gene expression in the cerebellum and cerebral cortex occurs on PND10 and PND20, respectively (*57*).

Interaction of Retinoic Acid and Thyroid Systems during Brain Development

The interaction of the vitamin A and thyroid systems is important for eliciting thyroid hormone action (*88*). Vitamin A (retinol) is obtained from the diet and passes through two enzymatic conversions to derive the bioactive metabolite RA. Like TR, RA receptors are members of the steroid/retinol/vitamin D/thyroid hormone nuclear receptor superfamily. There are two classes of RA receptors: the retinoic acid receptor (RAR), which binds most naturally occurring RAs, and RXR, which binds only 9-*cis*-RA. These RA receptor isoforms elicit RA action by binding as homodimers or heterodimers with each other. Thyroid receptors can heterodimerize with both classes of RA receptors (*18*).

However, TR preferentially heterodimerizes with the RXR, which often results in more effective activation of the corresponding thyroid or RA response element than a TR or RXR homodimer (*88*). In the rat, TSHβ gene expression is regulated by T_3 and RA via TR-RXR and RAR-RXR heterodimers, respectively (*89*). *In vitro* studies of rat optic nerve oligodendrocyte precursor cells determined that RA as well as thyroid hormone plays a role in promoting the differentiation of these cells into mature oligodendrocytes cultures, possibly through influence on the activity of transcription factor activator protein 1 (*90*). Koibuchi and Chin (*91*) reported that thyroid hormone accelerated the initiation of RA orphan receptor α expression in developing rat Purkinje cells in the hypothyroid rat cerebellum, suggesting that thyroid hormone may contribute to the Purkinje cell differentiation via interaction with the RA system.

Xenobiotic Interference with the Thyroid System

As mentioned in my introduction, the panelists at the Duke University workshop agreed that there were assays currently in use that could detect changes in thyroid hormone action and gland function (*1*). They identified a number of assays that could be used to screen chemicals for their potential to interfere with the thyroid system but emphasized that none of the assays were adequate as a first-tier screen. The assays were as follows: *a*) a TRH challenge, which tests the integrity of the hypothalamic–pituitary–thyroid axis by measuring TSH production following the administration of TRH; *b*) the use of a perchlorate discharge test to detect chemicals that inhibit the uptake of iodide by the thyroid gland; *c*) a quantification of thyroid peroxidase, the key enzyme in thyroid hormone production that assures the oxidation of iodide, the incorporation of iodide into the tyrosine residues on TBG, and the coupling of the di- and triiodotyrosyl residues on TBG to form thyroid hormone; *d*) a series of thyroid hormone competitive binding assays to quantify maternal serum-binding proteins, which can predict changes in fetal thyroid hormone concentrations; *e*) a measure of iodothyronine deiodinase activity of the three tissue specific enzymes (type I, II, III proteins) that facilitate the removal of iodine from thyroid hormone; and *f*) a measure of the rate of thyroid hormone glucuronidation using an *in vivo* exposure paradigm followed by *ex vivo* examination using hepatic microsomes. Glucuronidation of thyroid hormone, which is facilitated by the enzyme uridine diphosphate-glucuronyl transferase (UDP-GT), produces an inactive, excretable form of the hormone; under normal conditions, glucuronidation helps to maintain the proper free thyroid hormone levels.

Indeed, synthetic chemicals can disrupt nearly every step in the production and metabolism of thyroid hormone (*92,93*) (Table 1). Chemical interference with uptake of iodide by the thyroid gland and, more specifically with the sodium/iodide symporter (which facilitates the iodide uptake), can result in a decrease in the circulating levels of T_4/T_3 (*94,95*). Chemical exposure can also lead to a decrease in serum protein–bound iodide levels, perhaps largely due to inhibition of the thyroid peroxidase enzyme, which disrupts the normal production of thyroid hormone (*96–98*). The displacement of T_4/T_3 from the transport proteins (TBG, transthyretin, and albumin) may result in decreased ability of thyroid hormone to reach its target tissue and may facilitate the transport of the chemicals into the fetus (*92,99*). Chemical disruption of the T_4/T_3 metabolism can influence deiodinase, glucuronidase, and sulfatase activity, and may ultimately result in increased biliary elimination of T_4/T_3. Inhibition of the deiodinase enzymes can result in a decrease in T_3 available to elicit thyroid action at the tissue level (*100*). Conversely, deiodinase activity may increase in response to chemical exposure, either as a direct effect or in response to increased clearance of T_4/T_3 by the chemical stimulation of glucuronidase or sulfatase enzymes (*101,102*). Brucker-Davis (*93*) suggests that such increases in the metabolism and clearance of T_3 could result in a goiter as the thyroid gland increases production to maintain proper hormone levels. The list in Table 1 of 116 chemicals capable of disrupting normal thyroid hormone production, transport, and metabolism is by no means exhaustive; further discussion of the effects of disruption of these processes can be found in reviews by Brouwer et al. (*92*) and Brucker-Davis (*93*). There are many more chemicals that have effects on TSH and T_4/T_3 levels, and thyroid histopathology for which no mechanism has been tested (*93*). It is unlikely that these chemicals are working as T_4/T_3 agonists or antagonists at the level of TR binding, as no chemical tested thus far has demonstrated high affinity binding to the mammalian TR. (*103,104*).

Relatively few studies have evaluated the mechanism of action of thyroid-disrupting chemicals in the fetal/neonatal organism. Darnerud et al. (*105*) reported that developmental exposure to 4-OH-3,5,3′,4′-tetrachlorobiphenyl, a major metabolite of polychlorinated biphenyl (PCB) congener 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), binds to fetal and maternal transthyretin in mice on GD17; significant decrease in fetal T_4 (free and total) was reported. Aminotriazole inhibited the catabolism of T_4 to T_3 in renal primary cell cultures from 4 to 5 months of gestation in human fetuses, indicating an interference with type 1 iodothyronine deiodinase function in the kidney (*106*). *In utero* exposure to PCB congener 3,3′,4,4′,5,5′-hexachlorobiphenyl alone or in combination with PCB 77 increased type II deiodinase activity in whole-brain homogenates from fetal (GD20) and neonatal (PND7 and PND21) rats; total T_4 levels in plasma were decreased by both treatments (*107*). UDP-GT activity was increased in PND21 rat weanlings exposed to the PCB congeners PCB 77, PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl), or TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) on GD10 (*108*). The increase in UDP-GT activity was seen in the near absence of significant decreases in T_4 concentration on PND21 (*108*). Gestational exposure to Aroclor 1254 depressed UDP-GT activity in GD20 rat fetuses, while increasing the enzyme in PND21 rats (*109*). The total and free T_4 levels in GD20 fetuses were significantly suppressed by both levels of Aroclor 1254 exposure during development, whereas the total T_4 and total T_3 were significantly depressed on PND21 only by the highest dose of Aroclor 1254 (*109*). The ability of man-made chemicals to disrupt the thyroid system strongly suggests the possibility of chemical perturbation of thyroid-sensitive brain development (*92,110,111*).

Chemical perturbation also influences the interaction of the thyroid hormone and RA systems. In young adult rats, RA is displaced from its serum transport protein, retinol binding protein, and the transthyretin-retinol binding protein complex by PCB 77 (*112*). Similar effects by PCB 77 on binding to transport proteins and serum levels of T_4 have been reported (*99,105*). Subchronic exposure to 2,2′,3,3′,4,4′-hexachlorobiphenyl decreased kidney and liver levels of vitamin A and significantly lowered frontal cortex dopamine levels in rats dosed for 4 weeks after weaning (*113*). Reduced follicle size and papillary proliferation of the thyroid epithelium were noted in all treatment levels. Hallgren et al. (*114*) reported that polybrominated diphenyl ether (PBDE) and Bromokal (a PBDE mixture) given orally to adult rats and mice significantly depressed serum T_4. Bromokal also reduced vitamin A levels in the liver and induced UDP-GT activity at the highest

Table 1. Synthetic chemicals that interfere with the production, transport, and metabolism of thyroid hormone.

Thyroid mechanism and interfering chemical

Uptake of iodide by thyroid gland
- 2,4-D (*137*)
- 3-Amino-1,2,4-triazole (*138,139*)
- Aldrin (*140*)
- Amitrole (*141,142*)
- Aroclor 1254 (*143,145*)
- 1,2-Dihydroxybenzene (catechol) (*146*)
- 4-Chlororesorcinol (*146*)
- Clofentezine (*141*)
- *o*-Cresol (*146*)
- *p*-Cresol (*146*)
- Cythion (*96,147*)
- 1,3-Dihydroxynaphthalene (*146*)
- 1,5-Dihydroxynaphthalene (*146*)
- 2,3-Dihydroxynaphthalene (*146*)
- 2,7-Dihydroxynaphthalene (*146*)
- 2,4-Dihydroxybenzaldehyde (*146*)
- 2,4-Dihydroxybenzoic acid (*146*)
- Ethiozin (*141*)
- Ethylene thiourea (*141,148*)
- Fipronil (*141*)
- Hexachlorobenzene (*149,150*)
- Hexadrin (*147*)
- 4-Hexylresorcinol (*146*)
- 1,3,4-Trihydroxybenzene (hydroxyquinol) (*146*)
- Hydroxyquinol triacetate (*146*)
- Lead (*151*)
- Mancozeb (*152*)
- Mercuric chloride (*153,154*)
- 3-Methylcholanthrene (*143,155*)
- Methylmercuric chloride (*154*)
- Methylparathion (*156*)
- 2-Methylresorcinol (*146*)
- Mull-Soy (*157*)
- Nabam (*140*)
- 5-Methylresorcinol (orcinol) (*146*)
- Pendimethalin (*141*)
- Pentachloronitrobenzene (*141*)
- Phenobarbital (*143*)
- Phenol (*146*)
- 1,3,5-Trihydroxybenzene (phloroglucinol) (*146*)
- Polybrominated biphenyls (*158*)
- Pregnenolone-16α-carbonitrile (*143*)
- Propylthiouracil (*139,158*)
- 1,2,3-Trihydroxybenzene (pyrogallol) (146)
- Pyrimenthanil (*141*)
- 1,3-Dihyroxybenzene (resorcinol) (*146*)
- *o*-Hydroxybenzyl alcohol (saligenin) (*146*)
- Selenium (*151*)
- Thiocyanate (*141*)

Sodium/iodide symporter
- Perchlorate (*94,159*)
- Perrhenate (*159*)

Serum protein–bound iodide level
- 2,4-D (*137*)
- 2,4-Dinitrophenol (*96*)
- 3-Methylcholanthrene (*155*)
- Amitrole (*142*)
- Aroclor 1254 (*144*)
- Cythion (*95,147*)
- Malathion (*160*)
- Mancozeb (*152*)
- Mercuric chloride (*153*)
- *o,p*′-DDD (*161,162*)
- Hexadrin (147)

Thyroid peroxidase action—general information
- Amitrole (*141*)
- Ammonia (*163*)
- Ethylene thiourea (*141*)
- Fipronil (*141*)
- Mancozeb (*141*)
- 4,4′-Methylenedianiline (*141*)
- Thiocyanate (*141*)

Thyroid peroxidase action—oxidation of iodide
- Aminotriazole (*97,164*)
- Ammonia (*163*)
- Cadmium chloride (*163,165*)
- Endosulfan (*166*)
- Ethylene thiourea (*98*)
- 1,2,3,4,5,6-Hexachlorocyclohexane (lindane) (*167*)
- Malathion (*167*)
- Mancozeb (*152*)
- Mercury chloride (*165*)
- Methamizole (*97*)
- Polybrominated biphenyls (*158*)
- Thiourea (*166*)

Thyroid peroxidase action—iodination of tyrosine
- Polybrominated biphenyls (*158*)

Binding to thyroglobulin
- *o,p*′-DDD (*161*)
- Pentachlorophenol (*168*)

Binding to transthyretin
- Bromoxynil (3,5-bibromo-4-hydroxybenzonitril) (*99*)
- 4-(Chloro-*o*-tolyloxy) acetic acid (*99*)
- 4-(4-Chloro-2-methylphenoxy) butyric acid (*99*)
- Chlorophenol (*99,169*)
- Chloroxuron (*99*)
- 2,4-D (*99*)
- 2,4-Dichlorophenoxybutric acid (*99*)
- Dioxtylphthalate (*99*)
- *o,p*′-DDD (99)
- *p,p*′-DDD (*99*)
- 2,3-Dichlorophenol (*99,169*)
- 2,4-Dichlorophenol (*99*)
- 2,6-Dichlorophenol (*99,169,170*)
- 2-(2,4-Dichlorophenoxy) propionic acid [dichloroprop] (*99*)
- 1,1,1-Trichloro-2,2-bis (chlorophenol) ethanol [difocol] (*99*)
- 2,4-Dinitrophenol (*99*)
- 2,4-Dinitro-6-methylphenol (*99*)
- Ethyl-bromophos (*99*)
- Ethyl-parathion (*99*)
- 2-(2,4,5-Trichlorophenoxy) propionic acid [fenoprop] (*99*)
- Hexachlorobenzene (*99*)
- Hexachlorophene (*99,169*)
- 2-Hydroxybiphenyl (*99*)
- 4-Hydroxybiphenyl (*99,169*)
- Lindane (*99*)
- Linuron (*99*)
- Malathion (*99*)
- Pentachlorophenol (*99,169,170*)
- Phenol (*169*)
- Pyrogallol (*99*)
- 2,4,5-Trichlorophenoxyacetic acid (*99*)
- 1,4-Tetrachlorophenol (*99,170*)
- PCB-77 (*99,105,169*)
- Trichloroacetic acid (*99*)
- Trichlorobenzene (*99*)
- 2,3,4-Trichlorophenol (*170*)
- 2,4,5-Trichlorophenol (*99,169,170*)
- 2,4,6-Trichlorophenol (*99,170*)
- 2,4,5-Trichlorophenoxyacetic acid methyl ester (*99*)

Binding to albumin
- Pentachlorophenol (*169*)

Catabolism of T_4 or T_3: type I or II 5′-deiodinase
- 3,3′,4,4′,5,5′-Hexachlorobiphenyl (*107*)
- 3-Methylcholanthrene (*171,172*)
- Aminotriazole (*106*)
- Amiodarone (*94,172*)
- Aroclor 1254 (*109*)
- Cadmium chloride (*173*)
- Diphenylthiohydantoin (*141,172*)
- Dimethoate (*100,174*)
- Fenvalerate (*175,176*)
- Hexachlorobenzene (*102*)
- Lead (*177*)
- Phenobarbital (*172*)
- Propylthiouracil (*172*)
- PCB 77 (*107,171*)
- TCDD (*171,178*)

Glucuronidation of T_4/T_3
- Acetochlor (*141*)
- Aroclor 1254 (*109,143–145,179*)
- 3,4-Benzpyrene (*180*)
- Clofentenzine (*141*)
- Clofibrate (*141*)
- DDT (*144*)
- Fenbuconazole (*141*)
- 3,3′,4,4′,5,5′-Hexabromobiphenyl (*101*)
- Hexachlorobenzene (*102,183*)
- 2,3,3′,4,4′,5-Hexachlorobiphenyl (*182*)
- 3,3′,4,4′,5,5′-Hexachlorobiphenyl (*107*)
- 3-Methylcholanthrene (*141,143,155,171,179*)
- Pendimethalin (*141*)
- PCB 126 (*108,182*)
- Phenobarbital (*141,143,172,180,181,183*)
- Polybrominated biphenyls (*184*)
- PCBs (*141*)
- Pregnenolone-16α-carbonitrile (*141,143,179*)
- Promadiamine (*141*)
- Pyrimethanil (*141*)
- PCB 77 (*108,171*)
- TCDD (*108,141,178,182*)
- Thiazopyr (*141*)

Catabolism and biliary elimination of T_4/T_3 in the liver
- Aroclor 1254 (*144,145*)
- 3,4-Benzopyrene (*180*)
- DDT (*144*)
- Hexachlorobenzene (*102*)
- 3-Methylcholanthrene (*155*)
- Phenobarbital (*180,183*)
- Polybrominated biphenyls (*184*)

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane.

dose in the rats; similar results were found for 2,3,3′,4,4′-pentachlorobiphenyl and Aroclor 1254 (*114*). Further research is needed to better understand how disruption of the vitamin A system may influence the thyroid system and brain development.

Thyroid Sensitivity of Nervous System Derivatives

There are several nervous system derivatives influenced by thyroid hormone. Thyroid hormone receptor β2 isoform has been identified in the outer nuclear layer of the mouse retina, which contains the developing photoreceptors (*115*) and is thought to play a role in committing the photoreceptor progenitor cells to becoming green cone photoreceptor cells. The pacemaker cells of the mammalian heart contain TRα1 (*42,116*) and T_3 may influence pacemaker activity by increasing the ion (Na^+-Ca^{2+}) activity (*117*). The following section will discuss the thyroid-sensitive nature of the development and function of the cochlea. Impaired function of the cochlea as well as of the eye and heart have been reported in laboratory studies as a result of thyroid perturbation during development and could be useful end points in detecting thyroid disruption.

Cochlear Development

Hearing loss is commonly associated with thyroid disorders in humans, including congenital hypothyroidism (*118,119*), iodide deficiency (*120*), resistance to thyroid hormone (*121*), and Pendred syndrome (*122*). Likewise, deafness is reported in the hyt/hyt mouse model with congenital hypothyroidism (*123*). TRβ1 and TRβ2 mRNA are expressed in the rat fetus as early as GD12.5 in the region of the otic vesicle that gives rise to the cochlea (*35*). Auditory function is mediated specifically by TRβ, as evidenced by hearing loss in mice lacking a functional TRβ, whereas mice deficient in TRα have normal hearing (*124,125*). TRβ is necessary for development of cochlear function; mice deficient in TRβ exhibit delayed expression of a key potassium conductance necessary for the maturation of the cochlear inner hair cells (*125*). Thyroid hormones acting through TRβ also influence hearing by initiating myelinogenesis of the cochlea and vestibulocochlear nerve (cranial nerve VIII) prior to the onset of hearing (*56*). In rats, the critical periods of thyroid-sensitive ear development extend from at least GD18 (the onset of fetal thyroid gland function) through PND18 (the period of outer hair cell synaptogenesis in the cochlea) (*126–128*). Brucker-Davis et al. (*121*) estimate the critical period of thyroid-sensitive cochlear development in humans extends from the close of the first trimester through the first month of life.

Hypothyroidism caused by developmental exposure to thyroid-disrupting chemicals can also cause hearing loss. Adult rats developmentally exposed to propylthiouracil (*129*), methimazole (*130*), or Aroclor 1254 (*131*) have significantly elevated auditory thresholds (i.e., less sensitive hearing) relative to controls. Hearing impairment caused by developmental exposure to Aroclor 1254 was identified as cochlear and/or auditory nerve dysfunction (*132*). It was due, in part, to a decrease in the abundance of outer hair cells in the upper middle turn (responsible for detection of lower frequency sounds) as well as the apical turn of the cochlea in rats (*133*). In a subsequent experiment, postnatal administration of T_4 partially blocked low-frequency hearing loss in rats developmentally exposed to Aroclor 1254 (*134*). With regard to human exposure, Chao et al. (*135*) compared children in Yucheng, Taiwan, who were born to women exposed to PCB- and polychlorinated dibenzofuran–contaminated rice oil with control children whose mothers were not exposed. Forty-four percent of children born to exposed mothers had abnormal middle ear morphology and/or impaired sound conductance compared with 18.8% of the controls. The identification of noninvasive methods of detecting thyroid system disruption of nervous systems end points such as impaired auditory thresholds may provide an important tool in laboratory and epidemiologic studies.

Summary

By superimposing the development of the thyroid system onto the chronology of brain development, it is evident that thyroid hormone may influence brain structure and function during the earliest stages of development in humans and rodents. Further investigation is needed to better understand the developmental patterns of thyroid-sensitive genes as well as their effects on brain development. Future versions of the timeline should also include development of the brain beyond birth and weaning, as many of the developmental processes continue well into adolescence in humans (*48*). In addition, species differences in the timing of brain development should be considered in the identification of thyroid-sensitive brain processes and developmental periods in mammals (*136*) and other vertebrates. Most important, as synthetic chemicals can interfere with nearly every step in thyroid system function, more research should be targeted at understanding how thyroid-disruptive chemicals may impact normal brain development and functioning. By increasing the knowledge of thyroid hormone influence on brain development and how chemicals can disrupt thyroid system function, scientists can design more effective screens and assays to test chemicals for their possible impact on the thyroid system and downstream brain effects.

References and Notes

1. DeVito M, Crofton K, McMaster S. Screening Methods for Chemicals That Alter Thyroid Hormone Action, Function and Homestasis. EPA 600-R-98-057. Durham, NC:U.S. Environmental Protection Agency, 1997.
2. Fisher DA. Fetal thyroid function: diagnosis and management of fetal thyroid disorders. Clin Obstet Gynecol 40(1):16–31 (1997).
3. Shepard TH. Onset of function in the human fetal thyroid: biochemical and radioautographic studies from organ culture. J Clin Endocrinol Metab 27(7):945–958 (1967).
4. Thorpe-Beeston JG, Nicolaides KH, McGregor AM. Fetal thyroid function. Thyroid 2:207–217 (1992).
5. Köhrle J. Thyroid hormone metabolism and action in the brain and pituitary. Acta Med Austriaca 27(1):1–7 (2000).
6. Glinoer D, Delange F. The potential repercussions of maternal, fetal, and neonatal hypothyroxinemia on the progeny. Thyroid 10(10):871–887 (2000).
7. Contempré B, Jauniaux E, Calvo R, Jurkovic D, Campbell S, Morreale de Escobar G. Detection of thyroid hormones in human embryonic cavities during the first trimester of pregnancy. J Clin Endocrinol Metab 77(6):1719–1722 (1993).
8. Kaplan MM. The maternal thyroid and parathyroid glands. In: Maternal and Fetal Endocrinology, 2nd ed (Tulchinsky D, Little AB, eds). Philadelphia:W.B. Saunders, 1994;131–149.
9. Vulsma T, Gons MH, De Vijlder JJM. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to total organification defect or thyroid agenesis. N Eng J Med 321(1):13–16 (1989).
10. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341(8):549–555 (1999).
11. Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijder JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol 50:149–155 (1999).
12. Klein RZ, Sargent JD, Larsen PR, Waisbren SE, Haddow JE, Mitchell ML. Relation of severity of maternal hypothyroidism to cognitive development of offspring. J Med Screen 8:18–20 (2001).
13. Leneman M, Buchanan L, Rovet J. *Where* and *what* visuospatial processing in adolescents with congenital hypothyroidism. J Int Neuropsychol 7:556–562 (2001).
14. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the human fetal brain. Endocrinology 114(2):677–679 (1984).
15. Ferreiro B, Bernal J, Goodyer CG, Branchard CL. Estimation of nuclear thyroid hormone receptor saturation in human fetal brain and lung during early gestation. J Clin Endocrinol Metab 67(4):853–856 (1988).
16. Oppenheimer JH, Swartz HL. Stereo specific transport of triiodothyronine from plasma to cytosol and from cytosol to nucleus in rat liver, kidney, brain, and heart. J Clin Invest 75:147–154 (1985).
17. Fisher DA, Klein AH. Thyroid development and disorders of thyroid function in the newborn. N Engl J Med 304(12):702–712 (1981).
18. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev 14(2):184–193 (1993).
19. Iskaros J, Pickard M, Evans I, Sinha A, Hardiman P, Ekins R. Thyroid hormone receptor gene expression in first trimester human fetal brain. J Clin Endocrinol Metab 85(7):2620–2623 (2000).

20. Kilby MD, Gittoes N, McCabe C, Verhaeg J, Franklyn JA. Expression of thyroid receptor isoforms in the human fetal central nervous system and the effects of intrauterine growth restriction. Clin Endocrinol 53:469–477 (2000).
21. Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. Mol Hum Reprod 3(7):555–562 (1997).
22. Fisher DA, Dussault JH, Sack J, Chopra IJ. Ontogenesis of hypothalamic-pituitary-thyroid function and metabolism in man, sheep, and rat. Recent Prog Horm Res 33:59–116 (1976).
23. Roti E. Regulation of thyroid-stimulating hormone (TSH) secretion in the fetus and neonate. J Endocrinol Invest 11:145–158 (1988).
24. Thliveris JA, Currie RW. Observations on the hypothalamo-hypophyseal portal vasculature in the developing human fetus. Am J Anat 157(4):441–444 (1980).
25. Winters AJ, Eskay RL, Porter JC. Concentration and distribution of TRH and LRH in the human fetal brain. J Clin Endocrinol Metab 39(5):960–963 (1974).
26. Aubert ML, Grumbach MM, Kaplan SL. The ontogenesis of human fetal hormones. IV: Somatostatin, luteinizing hormone releasing factor, and thyrotropin releasing factor in hypothalamus and cerebral cortex of human fetuses 10-22 weeks of age. J Clin Endocrinol Metab 44(6):1130–1141 (1977).
27. Kaufman MH, Bard JBL. Index 5.2: Table of mouse and rat development. In: The Anatomical Basis of Mouse Development (Kaufman MH, Bard JBL, eds). San Diego, CA:Academic Press, 1999;220–223.
28. Morreale de Escobar G, Pastor R, Obregon MJ, Escobar del Rey F. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. Endocrinology 117(5):1890–1900 (1985).
29. Woods RJ, Sinha AK, Ekins RP. Uptake and metabolism of thyroid hormones by the rat foetus in early pregnancy. Clin Sci (London) 67(3):359–363 (1984).
30. Versloot PM, Schröder-van der Elst JP, van der Heide D, Boogerd L. Effects of marginal iodine deficiency on thyroid hormone production, distribution and transport in nonpregnant and near-term pregnant rats. Eur J Endocrinol 138:713–718 (1998).
31. Calvo R, Obregon MJ, Ruiz de Oña C, Escobar del Rey F, Morreale de Escobar G. Congenital hypothyroidism, as studied in rats: crucial role of maternal thyroxine but not of 3,5,3′-triiodothyronine in the protection of the fetal brain. J Clin Invest 86:889–899 (1990).
32. Obregon MJ, Mallol J, Pastor R, Morreale de Escobar G, Escobar del Rey F. L-Thyroxine and 3,5,3′-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. Endocrinology 114(1):305–307 (1984).
33. Dussault JH, Labrie F. Development of the hypothalamic-pituitary-thyroid axis in the neonatal rat. Endocrinology 97(5):1321–1324 (1975).
34. Perez-Castillo A, Bernal J, Ferreiro B, Pans T. The early ontogenesis of thyroid hormone receptor in the rat fetus. Endocrinology 117(6):2457–2461 (1985).
35. Bradley DJ, Towle HC, Young WS. Alpha and beta thyroid hormone receptor (TR) gene expression during auditory neurogenesis: evidence for TR isoform-specific transcriptional regulation in vivo. Proc Natl Acad Sci U S A 91(2):439–443 (1994).
36. Falcone M, Miyamoto T, Fierro-Renoy F, Macchia E, DeGroot LJ. Evaluation of the ontogeny of thyroid hormone receptor isotypes in rat brain and liver using an immunohistochemical technique. Eur J Endocrinol 130:97–106 (1994).
37. Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormone-dependent brain development. Endocr Rev 18(4):462–475 (1997).
38. Ferreiro B, Pastor R, Bernal, J. T3 receptor occupancy and T3 levels in plasma and cytosol during rat brain development. Acta Endocrinol (Copenh) 123(1):95–99 (1990).
39. Bradley DJ, Towle HC, Young WS III. Spatial and temporal expression of α- and β-thyroid hormone receptor mRNAs, including β_2-subtype, in the developing mammalian nervous system. J Neurosci 12(6):2288–2302 (1992).
40. Strait KA, Schwartz HL, Seybold VS, Ling NC, Oppenheimer JH. Immunofluorescence localization of thyroid hormone receptor protein β1 and variant α2 in selected tissues: cerebellar Purkinje cells as a model for β1 receptor-mediated developmental effects of thyroid hormone in brain. Proc Natl Acad Sci U S A 88(9):3887–3891 (1991).
41. Leonard JL, Farwell AP, Yen PM, Chin WW, Stula M. Differential expression of thyroid hormone receptor isoforms in neurons and astroglial cells. Endocrinology 135(2):548–555 (1994).
42. Forrest D, Vennstrom B. Functions of thyroid hormone receptors in mice. Thyroid 10(1):41–52 (2000).
43. Rodríguez-García M, Jolín T, Santos A, Pérez-Castillo A. Effect of perinatal hypothyroidism on the developmental regulation of rat pituitary growth hormone and thyrotropin genes. Endocrinology 136(10):4339–4350 (1995).
44. Oliver C, Eskay, RL, Porter JC. Developmental changes in brain TRH and in plasma and pituitary TSH and prolactin levels in the rat. Biol Neonate 37(3–4):145–152 (1980).
45. Burgunder JM, Taylor T. Ontogeny of thyrotropin-releasing hormone gene expression in the rat diencephalon. Neuroendocrinology 49(6):631–640 (1989).
46. Shambaugh GE, Kubek M, Wilber JF. Characterization of rat placental TRH-like material and the ontogeny of placental and fetal brain TRH. Placenta 4(4):329–337 (1983).
47. Strbak V, Greer MA. Acute effects of hypothalamic ablation on plasma thyrotropin and prolactin concentrations in the suckling rat: evidence that early postnatal pituitary-thyroid regulation is independent of hypothalamic control. Endocrinology 105(2):488–492 (1979).
48. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108(suppl 3):511–533 (2000).
49. Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14(1):83–144 (1993).
50. Gould E, Tanapat P, Hastings NB, Shors TJ. Neurogenesis in adulthood: a possible role in learning. Trends Cogn Sci 3(5):186–192 (1999).
51. Kornack DR, Rakic P. The generation, migration, and differentiation of olfactory neurons in the adult primate brain. Proc Natl Acad Sci U S A 98(8):4752–4757 (2001).
52. Herschkowitz N, Kagan J, Zilles K. Neurobiological bases of behavioral development in the first year. Neuropediatrics 28:296–306 (1997).
53. Lauder JM, Bloom FE. Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. II: Synaptogenesis. J Comp Neurol 163(3):251–264 (1975).
54. Swann JW, Smith KL, Brady RJ. Neural networks and synaptic transmission in immature hippocampus. Adv Exp Med Biol 268:161–171 (1990).
55. Sutor B, Luhmann HJ. Development of excitatory and inhibitory postsynaptic potentials in the rat neocortex. Perspect Dev Neurobiol 2(43):409–419 (1995).
56. Knipper M, Bandtlow C, Gestwa L, Köpschall I, Rohbock K, Wiechers B, Zenner H-P, Zimmermann U. Thyroid hormone affects Schwann cell and oligodendrocyte gene expression at the glial transition zone of the VIIIth nerve prior to cochlea function. Development 125:3709–3718 (1998).
57. Ibarrola N, Rodríguez-Peña A. Hypothyroidism coordinately and transiently affects myelin protein gene expression in most rat brain regions during postnatal development. Brain Res 752:285–293 (1997).
58. Dussault JH, Ruel J. Thyroid hormones and brain development. Ann Rev Physiol 49:321–334 (1987).
59. Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development—current perspectives. Endocr Rev 14(1):94–106 (1993).
60. Bernal J, Nunez J. Thyroid hormones and brain development. Eur J Endocrinol 133(4):390–398 (1995).
61. Koibuchi N, Chin WW. Thyroid hormone action and brain development. Trend Endocrinol Metab 11(4):123–128 (2000).
62. Thompson CC, Potter GB. Thyroid hormone action in neural development. Cereb Cortex 10:939–945 (2000).
63. Nicholson JL, Altman J. The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I: Cell proliferation and differentiation. Brain Res 44:13–23 (1972).
64. Xiao Q, Nikodem VM. Apoptosis in the developing cerebellum of the thyroid hormone deficient rat. Front Biosci 3:a52–a57 (1998).
65. Neveu I, Arenas E. Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. J Cell Biol 133(3):631–646 (1996).
66. Muller Y, Rocchi E, Lazaro JB, Clos J. Thyroid hormone promotes BCL-2 expression and prevents apoptosis of early differentiating cerebellar granule neurons. Int J Dev Neurosci 13(8):871–885 (1995).
67. Balazs R, Kovacs S, Teichgraber P, Cocks WA, Eayrs JT. Biochemical effects of thyroid deficiency on the developing brain. J Neurochem 15(11):1335–1349 (1968).
68. Alvarez-Dolado M, Ruiz M, Del Rio JA, Alcántara S, Burgaya F, Sheldon M, Nakajima K, Bernal J, Howell BW, Curran T, et al. Thyroid hormone regulates *reelin* and *dab*1 expression during brain development. J Neurosci 19(16):6979–6693 (1999).
69. Farwell AP, Dubord-Tomasetti SA. Thyroid hormone regulates the expression of laminin in the developing rat cerebellum. Endocrinology 140(9):4221–4227 (1999).
70. Farwell AP, Dubord-Tomasetti SA. Thyroid hormone regulates the extracellular organization of laminin on astrocytes. Endocrinology 140(11):5014–5021 (1999).
71. Eayrs JT. The cerebral cortex of normal and hypothyroid rats. Acta Anat 25:160–183 (1955).
72. Nicholson JL, Altman J. The effects of early hypo- and hyperthyroidism on the development of the rat cerebellar cortex. II: Synaptogenesis in the molecular layer. Brain Res 44(1):25–36 (1972).
73. Ruiz-Marcos A, Sanchez-Toscano F, Escobar del Rey F, Morreale de Escobar G. Severe hypothyroidism and the maturation of the rat cerebral cortex. Brain Res 162(2):315–329 (1979).
74. Gravel C, Hawkes R. Maturation of the corpus callosum of the rat. I: Influence of thyroid hormones on the topography of callosal projections. J Comp Neurol 291:128–146 (1990).
75. Rami A, Rabie A. Delayed synaptogenesis in the dentate gyrus of the thyroid-deficient developing rat. Dev Neurosci 12:398–405 (1990).
76. Thompson CC. Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a *hairless* homolog. J Neurosci 16(24):7832–7840 (1996).
77. Rodríguez-Peña A. Oligodendrocyte development and thyroid hormone. J Neurobiol 40(4):497–512 (1999).
78. Rakic P. Guidance of neurons migrating to the fetal monkey neocortex. Brain Res 33(2):471–476 (1971).
79. Sidman RL, Rakic P. Neuronal migration with special reference to developing human brain: a review. Brain Res 62 (1):1–35 (1973).
80. Mission J-P, Takahashi T, Caviness VS Jr. Ontogeny of radial and other astroglial cells in murine cerebral cortex. Glia 4:138–148 (1991).
81. Gressens P, Gofflot F, van Maele-Fabry G, Mission J-P, Gadisseux J-F, Evrard P, Picard JJ. Early neurogenesis and teratogenesis in whole mouse embryo cultures. Histochemical, immunocytological and ultrastructural study of the premigratory neuronal-glial units in normal mouse embryo and in mouse embryos influenced by cocaine and retinoic acid. J Neuropathol Exp Neurol 51:206–219 (1992).
82. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk sac, and which proliferates in the brain. Brain Res Dev Brain Res 117(2):145–152 (1999).
83. Schwab JM, Schluesener HJ, Seid K, Meyermann R. IL-16 is differentially expressed in the developing human fetal brain by microglial cells in zones of neuropoesis. Int J Dev Neurosci 19(1):93–100 (2001).
84. Martínez-Galan JR, Pedraza P, Santacana M, Escobar del Ray F, Morreale de Escobar G, Ruiz-Marcos A. Early effects of iodine deficiency on radial glial cells of the hippocampus of the rat fetus. A model of neurological cretinism. J Clin Invest 99(11):2701–2709 (1997).
85. McCall MA, Gregg RG, Behringer RR, Brenner M, Delaney CL, Galbreath EJ, Zhang CL, Pearce RA, Chiu SY, Messing A. Targeted deletion in astrocyte intermediate filament (*Gfap*) alters neuronal physiology. Proc Natl Acad Sci USA 93:6361–6366 (1996).
86. Lima FR, Gervais A, Colin C, Izembart M, Neto VM, Mallat M. Regulation of microglial development: a novel role for thyroid hormone. J Neurosci 21(6):2028–2038 (2000).
87. Verity AN, Campagnoni AT. Regional expression of myelin protein genes in the developing mouse brain: in situ hybridization studies. J Neurosci 21(2-4):238–248 (1988).

88. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. Cell 83(6):841–850 (1995).
89. Breen JJ, Hickok NJ, Gurr JA. The rat TSH-beta gene contains distinct response elements for regulation by retinoids and thyroid hormone. Mol Cell Endocrinol 131(2):137–146 (1997).
90. Barres BA, Lazar MA, Raff MC. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120:1097–1108 (1994).
91. Koibuchi N, Chin WM. ROR α gene expression in the perinatal rat cerebellum: ontogeny and thyroid hormone regulation. Endocrinology 139(5):2335–2341 (1998).
92. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health 14(1-2):59–84 (1998).
93. Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. Thyroid 8(9):827–856 (1998).
94. Wolff J. Perchlorate and the thyroid gland. Pharmacol Rev 50(1):89–105 (1998).
95. Ram RN, Joy KP, Sathyanesan AG. Cythion-induced histophysiological changes in thyroid and thyrotrophs of the teleost fish, *Channa punctatus* (Bloch). Ecotoxicol Environ Saf 17:272–278 (1989).
96. Goldberg RC, Wolff J, Greep RO. The mechanism of depression of plasma protein bound iodine by 2,4 dinitrophenol. Endocrinology 56:560–566 (1955).
97. Ealey PA, Henderson B, Loeveridge N. A quantitative study of peroxidase activity in unfixed tissue sections of the guinea-pig thyroid gland. Histochem J 16:111–122 (1984).
98. Marinovich M, Guizzetti M, Ghilardi F, Viviani B, Corsini E, Galli CL. Thyroid peroxidase as toxicity target for dithiocarbamates. Arch Toxicol 71(8):508–512 (1997).
99. van den Berg KJ, van Raaij JAGM, Bragt PC, Notten WRF. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. Arch Toxicol 65:15–19 (1991).
100. Maiti PK, Kar A. Dimethoate inhibits extrathyroidal 5′-monodeiodination of thyroxine to 3,3′,5-triiodothyronine in mice—the possible involvement of the lipid peroxidative process. Toxicol Lett 91(1):1–6 (1997).
101. Spear PA, Higueret P, Garcin H. Increased thyroxine turnover after 3,3′,4,4′,5,5′-hexabromobiphenyl injection and lack of effect on peripheral triiodothyronine production. Can J Physiol Pharmacol 68(9):1079–1084 (1990).
102. van Raaij JAGM, Kaptein E, Visser TJ, van den Berg KJ. Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats. Biochem Pharmacol 45(3):627–631 (1993).
103. Cheek AO, Kow K, Chen J, McLachlan JA. Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. Environ Health Perspect 107:273–278 (1999).
104. McKinney J, Fannin R, Jordan S, Chae K, Rickenbacher U, Pedersen L. Polychlorinated biphenyls and related compound interactions with specific binding sites for thyroxine in rat liver nuclear extracts. J Med Chem 30:79–86 (1987).
105. Darnerud PO, Morse D, Klasson-Wehler E, Brouwer A. Binding of a 3,3′,4,4′-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. Toxicology 103(1-3):105–114 (1996).
106. Ghinea E, Dumitriu L, Stefanovici G, Pop A, Oprescu M, Ciocirdia C. Action of some pesticides on T4 to T3 conversion in cultured kidney and liver cells in the presence or absence of cysteine. Rev Roum Med Endocrinol 24(3):157–166 (1986).
107. Morse DC, Koeter HBWM, Smits van Prooijen AE, Brouwer A. Interference of polychlorinated biphenyls in thyroid hormone metabolism: possible neurotoxic consequences in fetal and neonatal rats. Chemosphere 25(1-2):165–169 (1992).
108. Seo BW, Li MH, Hansen LG, Moore RW, Peterson RE, Schantz SL. Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. Toxicol Lett 75(3): 253–262 (1995).
109. Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). Toxicol Appl Pharm 136(2):269–279 (1996).
110. Porterfield SP. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 102(suppl 2):125–130 (1994).
111. Zoeller RT, Crofton KM. Thyroid hormone action in fetal brain development and potential for disruption by environmental chemicals. Neurotoxicology 21(6):935–945 (2000).
112. Brouwer A, van den Berg KJ. Binding of metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85:301–312 (1986).
113. Lecavalier P, Chu I, Yagminas A, Villeneuve DC, Poon R, Feeley M, Hakansson H, Ahlborg UG, Valli VE, Bergman A, et al.. Subchronic toxicity of 2,2′,3,3′,4,4′-hexachlorobiphenyl in rats. J Toxicol Environ Health 51(3):265–277 (1997).
114. Hallgren S, Sinjari T, Hakansson H, Darnerud PO. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch Toxicol 75:200–208 (2001).
115. Ng L, Hurley JB, Dierks B, Srinivas M, Salto C, Vennstrom B, Reh TA, Forrest D. A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat Genet 27:94–98 (2001).
116. Blange I, Drvota V, Yen PM, Sylven C. Species differences in cardiac thyroid hormone receptor isoforms protein abundance. Biol Pharm Bull 20(11):1123–1126 (1997).
117. Sun Z-Q, Ojamaa K, Nakamura TY, Artman M, Klein I, Coetzee WA. Thyroid hormone increases pacemaker activity in rat neonatal atrial myocytes. J Mol Cell Cardiol 33:811–824 (2001).
118. Bellman SC, Davies A, Fuggle PW, Grant DB, Smith I. Mild impairment of neuro-otological function in early treated congenital hypothyroidism. Arch Dis Child 74(3):215–218 (1996).
119. Debruyne F, Vanderschueren-Lodeweychx M, Bastijns P. Hearing in congenital hypothyroidism. Audiology 22(4):404–409 (1983).
120. Soriguer F, Millon MC, Munoz R, Mancha I, Siguero JPL, Aedo MJM, Gomez-Huelga R, Garriga MJ, Rojo-Martinez G, Esteva I, et al. The auditory threshold in a school-age population is related to iodine intake and thyroid function. Thyroid 10(11):991–999 (2000).
121. Brucker-Davis F, Skarulis MC, Pikus A, Ishizawar D, Mastroianni M-A, Koby M, Weintraub BD. Prevalence and mechanisms of hearing loss in patients with resistance to thyroid hormone. J Clin Endocrinol Metab 81(8):2768–2772 (1996).
122. Forrest D. Editorial: Deafness and goiter: molecular genetic considerations. J Clin Endocrinol Metab 81(8):2764–2767 (1996).
123. Li D, Henley CM, O'Malley BW Jr. Distortion product otoacoustic emissions and outer hair cell defects in the hyt/hyt mutant mouse. Hear Res 138(1-2):65–72 (1999).
124. Forrest D, Erway LC, Ng L, Altschuler R, Curran T. Thyroid hormone receptor β is essential for development of auditory function. Nat Genet 13:354–357 (1996).
125. Rusch A, Erway LC, Oliver D, Vennstrom B, Forrest D. Thyroid hormone receptor β-dependent expression of a potassium conductance in inner hair cells at the onset of hearing. Proc Natl Acad Sci U S A 95(26):15758–15762 (1998).
126. Hebert R, Langlois J-M, Dussault JH. Permanent defects in rat peripheral auditory function following perinatal hypothyroidism: determination of a critical period. Dev Brain Res 23:161–170 (1985).
127. Uziel S. Periods of sensitivity to thyroid hormone during the development of the organ of Corti. Acta Otolaryngol Suppl 429:23–27 (1986).
128. Freeman S, Cherny L, Sohmer H. Thyroxine affects physiological and morphological development of the ear. Hear Res 97(1-2):19–29 (1996).
129. Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. Toxicol Appl Pharmacol 135:67–76 (1995).
130. Knipper M, Zinn C, Maier H, Praetorius M, Rohbock K, Kopschall I, Zimmermann U. Thyroid hormone deficiency before the onset of hearing causes irreversible damage to peripheral and central auditory systems. J Neurophysiol 83(5):3101–3112 (2000).
131. Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduced circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 135:77–88 (1995).
132. Herr DW, Goldey ES, Crofton KM. Developmental exposure to Aroclor 1254 produces low-frequency alterations in adult rat brainstem auditory evoked responses. Fundam Appl Toxicol 33(1):120–128 (1996).
133. Crofton KM, Ding D, Padich R, Taylor M, Henderson D. Hearing loss following exposure during development to polychlorinated biphenyls: a cochlear site of action. Hear Res 144:196–204 (2000).
134. Goldey ES, Crofton KM. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. Toxicol Sci 45:94–105 (1998).
135. Chao WY, Hsu CC, Guo YLL. Middle-ear disease in children exposed prenatally to polychlorinated biphenyls and polychlorinated dibenzofurans. Arch Environ Health 52(4):257–262 (1997).
136. Clancy B, Darlington RB, Finlay BL. Translating developmental time across mammalian species. Neuroscience 105(1):7–17 (2001).
137. Florsheim WH, Velcoff SM. Some effects of 2,4-dichlorophenoxyacetic acid in thyroid function in the rat: effects on iodine accumulation. Endocrinology 71(1):1–6 (1962).
138. Alexander NM. Antithyroid action of 3-amino-1,2,4-triazole. J Biol Chem 234(1):148–150 (1959).
139. Mayberry WE. Antithyroid effects of 3-amino-1,2,4-triazole. Proc Soc Exp Biol Med 129(2):551–556 (1968).
140. Kuzan FB, Prahlad KV. The effects of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydroxy endo, exo-5,8-dimthionaphthalene (aldrin) and sodium ethylenebisdithiocarbomate (Nabam) on the chick. Poult Sci 54:1054–1064 (1975).
141. Hurley PM, Hill RN, Whiting RJ. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ Health Perspect 106:437–445 (1998).
142. Steinhoff D, Weber H, Mohr U, Boehme K. Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. Toxicol Appl Pharmacol 69:161–169 (1983).
143. Barter RA, Klaasen CD. Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. Toxicol Appl Pharmacol 128:9–17 (1994).
144. Bastomsky CH. Effects of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on biliary thyroxine excretion in rats. Endocrinology 95(4):1150–1155 (1974).
145. Bastomsky CH, Murthy PVN, Banovac K. Alterations in thyroxine metabolism produced by cutaneous application of microscope immersion oil: effects due to polychlorinated biphenyls. Endocrinology 98:1309–1314 (1976).
146. Arnott DG, Doniach I. The effect of compounds allied to resorcinol upon the uptake of radioactive iodine by the thyroid of the rat. Biochem J 50:473–479 (1952).
147. Singh H, Singh TP. Thyroid activity and TSH potency of the pituitary gland and blood serum in response to cythion and hexadrin treatment in the freshwater catfish, *Heteropneustes fossilis* (Bloch). Environ Res 22:184–189 (1980).
148. Freudenthal RI, Kerchner G, Persing R, Baron RL. Dietary subacute toxicity of ethylene thiourea in the laboratory rat. J Environ Pathol Toxicol 1:147–161 (1977).
149. Smith AG, Dinsdale D, Cabral JRP, Wright AL. Goiter and wasting induced in hamsters by hexachlorobenzene. Arch Toxicol 60:343–349 (1987).
150. Kleiman de Pisarev DL, del Carmen Rios de Molina M, San Martin de Viale LC. Thyroid function and thyroxine metabolism in hexachlorobenzene-induced porphyria. Biochem Pharmacol 35(5):817–825 (1990).
151. Goldman M, Dillon RD. Interaction of selenium and lead on several aspects of thyroid function in Pekin ducklings. Res Commun Chem Pathol Pharmacol 37(3):487–490 (1982).

152. Kackar R, Srivastava MK, Raizada RB. Studies on rat thyroid after oral administration of mancozeb: morphological and biochemical evaluations. J Appl Toxicol 17(6):369–375 (1997).
153. Goldman M, Blackburn P. The effect of mercuric chloride on thyroid function in the rat. Toxicol Appl Pharmacol 48:49–55 (1979).
154. Kawada J, Nishida M, Yoshimura Y, Mitani K. Effects of organic and inorganic mercurials on thyroidal functions. J Pharmacobio-Dyn 3:149–159 (1980).
155. Bastomsky CH, Papapetrou PD. The effect of methycholanthrene on biliary thyroxine excretion in normal and Gunn rats. J Endocrinol 56:367–273 (1973).
156. Lybeck H, Leppaluoto J, Aito H. The influence of an anticholinesterase, methylparathion, on the radioiodine uptake of the rat thyroid *in vivo* and *in vitro*. Ann Med Exp Biol Fenn 45(1):76–79 (1967).
157. Van Wyk JJ, Arnold MB, Wynn J, Pepper F. The effects of a soybean product on thyroid function in humans. Pediatrics 24:752–760 (1959).
158. Allen-Rowlands CF, Castracane VD, Hamilton MG, Seifter J. Effect of polybrominated biphenyls (PBB) on the pituitary-thyroid axis of the rat (41099). Proc Soc Exp Biol Med 166(4):506–514 (1981).
159. Eskandari S, Loo DD, Dai G, Levy O, Wright EM, Carrasco N. Thyroid Na^+/I^- symporter. Mechanism, stoichiometry, and specificity. J Biol Chem 272(43):27230–27238 (1997).
160. Balasubramanian K, Vijayan AP, Ananthanarayanan PH, Balasubramanian A. Effect of malathion on the thyroid function of male albino rats. IRCS Med Sci 14:1139–1140 (1986).
161. Marshall JS, Tompkins LS. Effect of o,p′-DDD and similar compounds on thyroxine binding globulin. J Clin Endocrinol 28:386–392 (1968).
162. Danowski TS, Sarver ME, Moses C, Bonessi JV. o-p′DDD therapy in Cushing′s syndrome and in obesity with Cushingoid changes. Am J Med 37:235–250 (1964).
163. Bhattacharya T, Bhattacharya S, Ray AK, Dey S. Influence of industrial pollutants on thyroid function in Channa punctatus (Bloch). Indian J Exp Biol 27:65–68 (1989).
164. Strum JM, Karnovsky MJ. Aminotriazole goiter. Lab Invest 24(1):1–12 (1971).
165. Ghosh N, Bhattacharya S. Thyrotoxicity of the chlorides of cadmium and mercury in rabbit. Biomed Environ Sci 5:236–240 (1992).
166. Sinha N, Lal B, Singh TP. Effect of endosulfan on thyroid physiology in the freshwater catfish, *Clarias batrachus*. Toxicology 67:187–197 (1991).
167. Yadav AK, Singh TP. Pesticide-induced impairment of thyroid physiology in the freshwater catfish, *Heteropneustes fossilis*. Environ Poll 43:29–38 (1987).
168. van Raaij JAGM, Frijters CMG, van den Berg KJ. Hexachlorobenzene-induced hypothyroidism: involvement of different mechanisms by parent compound and metabolite. Biochem Pharmacol 46(8):1385–1391 (1993).
169. van den Berg KJ. Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. Chem Biol Interact 76:63–75 (1990).
170. den Besten C, Vet JJRM, Besselink HT, Kiel GS, Beems R, van Bladeren PJV. The liver, kidney, and thyroid toxicity of chlorinated benzenes. Toxicol Appl Pharmacol 111:69–81 (1991).
171. Visser TJ, Kaptein E, van Toor H, van Raaij JAGM, van den Berg KJ, Joe CTT, van Engelen JGM, Brouwer A. Glucuronidation of thyroid hormone in rat liver: effects of *in vivo* treatment with microsomal enzyme inducers and *in vitro* assay conditions. Endocrinology 133(5):2177–2186 (1993).
172. de Sandro V, Chevrier M, Boddaert A, Melcion C, Cordier A, Richert L. Comparison of the effects of propylthiouracil, amiodorane, diphenylhydantoin, phenobarbital, and 3-methylchloanthrene on hepatic and renal T4 metabolism and thyroid gland function in rats. Toxicol Appl Pharmacol 111:263–278 (1991).
173. Yoshida K, Sugihira N, Suzuki M, Sakurada T, Saito S, Yoshinaga K, Saita H. Effect of cadmium on T4 outer ring monodeiodination by rat liver. Environ Res 42:400–405 (1987).
174. Karabelyos CS, Szeberenyi SZ, Csaba G. Effect of allylestrenol exposure during pregnancy on the activity of microsomal enzyme system (PSMO) of rat in the F1 and F2 generations. Acta Physiol Hung 82(1):15–22 (1994).
175. Maiti PK, Kar A, Gupta P, Chaurasia SS. Loss of membrane integrity and inhibition of type I iodothyronine 5′-monodeiodinase activity by fenvalerate in female mouse. Biochem Biophys Res Commun 214(3):905–909 (1995).
176. Maiti PK, Kar A. Dual role of testosterone in fenvalerate-treated mice with respect to thyroid function and lipid peroxidation. J Appl Toxicol 17(2):127–131 (1997).
177. Chaurasia SS, Gupta P, Kar A, Maiti PK. Lead induced thyroid dysfunction and lipid peroxidation in the fish *Clarias batrachus* with special reference to hepatic type I-5′-monodeiodinase activity. Bull Environ Contam Toxicol 56(4):649–654 (1996).
178. Schuur AG, Boekhorst FM, Brouwer A, Visser TJ. Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone turnover in male Sprague-Dawley rats. Endocrinology 138(9):3727–3734 (1997).
179. Barter RA, Klaassen CD. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroid mechanism. Toxicol Appl Pharmacol 113:36–42 (1992).
180. Goldstein JA, Taurog A. Enhanced biliary excretion of thyroxine glucuronide in rats pretreated with benzpyrene. Biochem Pharmacol 17:1049–1065 (1968).
181. Rozman K, Gorski JR, Rozman P, Parkinson A. Reduced serum thyroid hormone levels in hexachlorobenzene-induced porphyria Toxicol Lett 30:71–78 (1986).
182. Van Birgelen APJM, Smit EA, Kampen IM, Groeneveld CN, Fase KM, Van der Kolk J, Poïger H, Van den Berg M, Koeman JH, Brouwer A. Subchronic effects of 2,3,7,8-TCDD of PCBs on thyroid hormone metabolism: use in risk assessment. Eur J Pharmacol-Environ Toxicol Pharmacol 293(1):77–85 (1995).
183. McClain RM, Levin AA, Posch R, Downing JC. The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. Toxicol Appl Pharmacol 99(2):216–228 (1989).
184. Ringer RK, Polin D. The biological effects of polybrominated biphenyls in avian species. Fed Proc 36(6):1894–1898 (1977).